This response is submitted to address the Examiner's outstanding issues and place the application in condition for allowance or in better form for consideration on Appeal.

REMARKS

As an initial matter, the telephone interview extended by Examiners Paras and Jill Martin on March 13, 2001, is greatly appreciated.

The following remarks encompass the arguments set forth by the undersigned in the interview, in regard to the Examiner's outstanding final rejections of claims 1-8 and 10-21 under 35 U.S/.C. 103(a) over Lavitrano taken with Kuretake.

In the Office Action, the Examiner maintains that both "live" and "dead" sperm are functionally equivalent with regard to the ability to fertilize oocytes. The Examiner incredibly maintains that "... ***'dead' sperm*** *are able to transfer DNA into an unfertilized oocyte* ***to create a transgenic non-human animal"***. Applicants assert that, **until the present invention**, there is absolutely no teaching or suggestion in the prior art that **dead sperm**[1] are able to **transfer exogenous transgene DNA** into an unfertilized oocyte to create a transgenic animal! Rather, the constant teaching of the prior art is that **live sperm, not dead sperm**, are necessary to transfer exogenous transgene DNA to an oocyte to create a transgenic animal. (See, *e.g.*, Lavitrano page 717, column 2, Results, last line paragraph 1, together with the Examiner's cite at page 721, column 1, Discussion, first paragraph). Applicants respectfully request that the Examiner provide prior art to support this allegation, in order to maintain it.

The Examiner alleges that applicants have not addressed the issue of whether or not "an increase in the rate of fertilization would also increase the rate of transgenesis" as a motivation for combining Lavitrano and Kuretake. On the contrary, applicants fully addressed this issue in the Amendment mailed November 22, 2000, and respectfully suggest that perhaps the invention is misunderstood in the Office Action. Applicants respectfully point out that, in the present invention,

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[1] The term "dead sperm" means that the plasma membrane has been damaged but that the nucleus has retained genetic integrity and is competent to fertilize an oocyte resulting in embryos and/or live offspring. Membrane-disrupted and demembranated sperm are "dead" according to the foregoing definition.

the transgene DNA transferred by dead sperm into unfertilized oocytes is exogenous DNA acquired by the dead sperm upon incubation of the transgene with the dead sperm prior to their injection into the unfertilized oocyte. The sperm by which the exogenous DNA is transferred are not from an animal that is already transgenic for the transgene. Therefore, the sperm themselves do not originally contain the desired transgene. Since neither Kuretake nor Lavitrano, alone or in combination, teach or suggest that dead sperm can take up exogenous DNA and transfer a desired transgene into an unfertilized oocyte resulting in a transgenic embryo or a live transgenic offspring, any method employed to increase the rate of fertilization (such as that taught by Kuretake) is immaterial to the present invention. (See further discussion below)

To further differentiate the present invention from the teachings of Lavitrano and/or Kuretake, the Examiner is kindly requested to refer to **Figure 1** of the Specification. This Figure illustrates a plasma membrane-intact sperm (A), a detergent-treated sperm (B), a freeze-thawed sperm (C) and a rehydrated freeze-dried sperm (D). The Examiner is requested to note that the membrane-disrupted sperm illustrated in (B), (C) and (D) have totally disrupted or missing plasma membranes. The sperm heads are exposed and perinuclear material remains attached to the sperm heads. These are the membrane-disrupted and/or demembranated sperm heads used to transfer exogenous transgene DNA into oocytes in the claims of the present invention. By the invention, the membrane-disrupted or demembranated sperm head is incubated with exogenous nucleic acid for a period of time sufficient for the exogenous nucleic acid to become associated with submembranal structures (*e.g.*, perinuclear material and/or nuclear DNA) of the sperm head. The exogenous nucleic acid and the sperm head are then co-inserted into the unfertilized oocyte, resulting in a transgenic embryo.

In contrast to the allegations of the Examiner, neither Lavitrano nor Kuretake, alone or in combination, teach or suggest the present invention. At most, Lavitrano teaches mixing live **membrane-intact** sperm with transgene DNA and adding oocytes to the mixture. The live sperm take up the transgene within an incubation period of about 30 minutes and, upon the addition of the oocytes, the treated sperm penetrate and fertilize the oocytes, to produce transgenic embryos. This is an *in vitro* fertilization (IVF) method employing live motile sperm. Applicants respectfully reiterate the arguments presented in the Amendment mailed 11/22/00. There is no question that Lavitrano is describing ***membrane-intact*** spermatozoa. Indeed other, later investigators have disclosed a model

of the process through which exogenous DNA binds to ***live*** ejaculated spermatozoa and is internalized and penetrates the sperm nucleus. (See, *e.g.*, Spadafora, C. "Sperm cells and foreign DNA: a controversial relation", BioEssays 20:955-964, 1998, especially pages 957-959, Table 1 and Figure 3; Gandolfi, F. Sperm-Mediated Transgenesis, Theriogenology 53: 127-137, 2000, especially pages 128-130, paragraph 2. These references were submitted previously). In particular, the model teaches that, in order to associate with a sperm nucleus, the exogenous foreign DNA requires binding to a DNA-binding protein (DBP) located on ***the surface of the sperm plasma membrane***. The binding activates the mechanism of internalization of a ***DNA/DBP complex*** by association with CD4 molecules (on the surface of the sperm plasma membrane) to form a ***DNA/DBP/CD4*** complex ***which then penetrates the nucleus, followed by dissociation at the nuclear matrix, releasing the exogenous DNA in close contact with the sperm chromosomal DNA***. Applicants submit that each of these active events clearly requires a ***live*** spermatozoa having ***intact plasma membrane proteins*** that actively internalize the exogenous DNA and that such proteins are not active in the membrane-disrupted or demembranated spermatozoa of the present invention. Further, Lavitrano teaches that fixation of live sperm with formaldehyde before incubation with labeled exogenous DNA resulted in negligible uptake of the DNA, suggesting that ***only living sperm cells*** are able to take up exogenous DNA (see Lavitrano, page 717, right column, last 13 lines). Thus, the active transport model described above supports the teachings of Lavitrano. However, neither Lavitrano, Spadafora nor Gandolfi teach or suggest that **membrane-disrupted** or **demembranated** sperm heads can take up exogenous DNA and that insertion of the treated sperm heads can transfer the exogenous DNA into an oocyte resulting in the formation of a transgenic embryo and, optionally, a transgenic live offspring. Rather, Lavitrano, Spadafora and Gandolfi teach against the present invention because their method requires a membrane-intact sperm.

Kuretake teaches intracytoplasmic sperm injection (ICSI). That is, Kuretake teaches that membrane-disrupted (by freeze-thawing) or detergent-treated sperm heads are capable of fertilizing oocytes when injected directly into the ooplasm, resulting in the development of ordinary embryos. There is absolutely no teaching or suggestion by Kuretake that these membrane-disrupted sperm heads can take up exogenous DNA, let alone that co-insertion of the exogenous DNA with membrane-disrupted or demembranated sperm heads results in **transgenic embryos** or transgenic

animals. Therefore, applicants respectfully assert that there would be no motivation to combine the teachings of Lavitrano that live membrane-intact sperm can take up exogenous DNA (such as by an active transport model described by Spadafora and/or Gandolfi) with the teachings of Kuretake that membrane-disrupted sperm heads can fertilize oocytes upon insertion of the heads into unfertilized oocytes.

The Examiner alleges that disruption of membranes increases the rate of fertilization by sperm. This teaching by Kuretake refers to the practice of damaging sperm tails immediately prior to injecting fresh sperm into oocytes. The Examiner then attempts to correlate an increase in the rate of fertilization as a means of increasing the rate of transgenesis. The Examiner appears to be using the argument that live sperm incubated with exogenous DNA would take up the DNA as taught by Lavitrano, and then, immediately prior to injection of the live sperm into oocytes (by ICSI), the tails would be damaged as taught by Kuretake, thus increasing the rate of fertilization and the rate of transgenesis. Applicants respectfully assert that, if this is indeed the argument that the Examiner is using, then the invention is misunderstood in the Office Action.

In particular, in the present invention, the sperm heads are membrane-disrupted or demembranated **prior to**, not after, incubation with the exogenous nucleic acid. Unexpectedly, and in contrast with the constant teachings of the prior art that intact membranes were necessary for uptake of exogenous nucleic acid, the exogenous nucleic acid bound irreversibly to submembranal structures associated with the sperm head and fertilization and transgenesis occurred when the sperm head and exogenous nucleic acid were co-inserted into an unfertilized oocytes. This totally unexpected finding is not taught or suggested by any of the prior art, especially not by Lavitrano or Kuretake, alone or in combination.

In view of the foregoing, applicants respectfully submit that Lavitrano taken with Kuretake does not teach or suggest the present invention. Withdrawal of the rejections under 35 U.S.C. 103(a) is respectfully requested.

Conclusion

Applicants believe that this response represents an accurate presentation of the discussion during the telephone interview with the Examiners. If there are inadvertent errors, the Examiner is respectfully requested to contact the undersigned.

Applicants submit that this application is in condition for allowance, and an early favorable response is respectfully requested.

Respectfully submitted

Barbara E. Arndt

Barbara E. Arndt, Ph.D.
Reg. No. 37,768

Jones, Day, Reavis & Pogue
North Point
901 Lakeside Avenue
Cleveland, Ohio 44114
(216) 586-7575